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THE MODULATION OF REWARD TO NICOTINE AND ETHANOL BY SEX AND STAGE OF EXPOSURE

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THESIS

Submitted in Partial Fulfillment of the
Requirements for the Degree of

Master of Arts

Psychology

The University of New Mexico
Albuquerque, New Mexico

May, 2017

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ABSTRACT

Tobacco and alcohol are among the most widely used and abused drugs in America, resulting in disastrous health consequences and a massive resource drain on society. Nicotine (the primary reinforcing component in tobacco) and alcohol are often used together, though there is limited research on exposure to both drugs at the same time. The present study attempted to fill this gap in knowledge by examining the reward for a cocktail of nicotine and alcohol in male and female Long-Evans rats with differing histories of drug exposure. The conditioned place preference paradigm was used to examine the effects of sex as well as the different histories of prenatal and/or adolescent drug exposure on reward for the cocktail. There was a main effect of sex on reward, with males showing a conditioned place preference for the cocktail and females showing no preference. Additional measures of locomotor activity induced by the drug cocktail differed depending on adolescent nicotine exposure, with rats having a previous history of nicotine exhibiting greater total distance travelled after receiving the cocktail. Results of the study indicate a possible moderating role of nicotine with alcohol co-exposure, and suggest that future studies should modify the exposure paradigm to better examine this potential role.

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Introduction

Alcohol and tobacco are among the most widely used and abused drugs in the US. In 2014 the National Health Interview Survey (NHIS) and Centers for Disease Control (CDC) estimated that 16.8% of adults in the US smoke tobacco; a number that totals more than 40 million people. That same year, a survey by the Substance Abuse and Mental Health Services Administration (SAMHSA) found that 71.0% of all participants reported drinking in the last year and 24.7% reported binge drinking in the last month. In 2012 the National Institute of Health reported that over 7% of Americans were classified as having an alcohol use disorder; this finding included over 800,000 adolescents. The negative health impacts of consuming alcohol and tobacco are staggering. In the US alone, approximately 88,000 people die annually from causes attributed to alcohol use (CDC, 2014) and 480,000 people die from diseases associated with tobacco use (Surgeon General, 2014). As cigarettes and alcohol are becoming more available worldwide, deaths attributed to these drugs are also rising. The World Health Organization (WHO) estimated that 3.3 million people died in 2014 because of alcohol consumption, and 6 million people died because of tobacco use; a figure that is climbing each year as smoking rates continue to rise in developing countries.

These high rates of alcohol and tobacco use cause a massive resource drain for society. In 2006, the CDC estimated that excessive drinking cost US citizens $223.5 billion annually and the Surgeon General (2014) estimated that tobacco smoking cost between $289 - $332.5 billion annually, depending on the individual outcomes attributed to smoking. By comparison, in 2011 The US Department of Justice estimated that all other forms of illicit drug use in the US cost a combined $193 billion. Though in the US

there are regulations in place to curtail alcohol and tobacco use (e.g., taxes, health warnings, age restrictions, etc.), these figures highlight the impact these drugs have had and continue to have on the individuals taking the drugs and our society as a whole.

Recently there has been a push to study nicotine—the primary reinforcing component in tobacco—and alcohol co-use. This initiative stems from findings that rates of smoking are positively correlated with alcohol dependence (Hughes et al, 2000; John et al, 2003; Falk et al, 2006). Indeed, Bobo and Husten (2000) reported that 37% of those classified as current smokers were also current drinkers, while less than 14% of respondents were current smokers that did not drink. DiFranza and Guerrera (1990) found that 83% of alcoholics were smokers; comparatively only 34% of non-alcoholics were smokers. More recently Room (2004) reported that depending on the country examined, 50-90% of those seeking treatment for alcohol use disorders were smokers. The high rate of alcohol and nicotine co-use, particularly for those whom have dependence on either or both drugs, highlights the need to further examine the interaction between alcohol and nicotine and the factors that drive it.

Research in humans and animals have studied the potential interactive effect by examining the effects of alcohol or nicotine pre-exposure on subsequent drug self-administration. In rodents, nicotine exposure prior to alcohol self-administration robustly increases the amount of alcohol ingested (Le et al, 2003; Dyr et al, 1999; Ericson et al, 2000; Doyon et al, 2013; Sharpe et al, 2002; Clark et al, 2001), a finding also detected in humans (Barrett et al, 2006; Kouri et al, 2004). Alcohol exposure prior to nicotine self-administration has been less consistent across animal research, with factors such as age of exposure, duration of exposure, dose and animal species affecting outcomes (Guavin et

al, 1993; Boutros et al, 2015; Mantella et al 2014; Darbra et al, 2004). Similarly, in humans the effect of alcohol on subsequent nicotine self-administration varies, with some studies finding no effect and others reporting an increase in nicotine self-administration (John et al, 2003; Zacny et al, 1996; Perkins et al, 2005).

In addition to self-administration experiments examining drug reinforcement, conditioned reward has been used as a way to study the interactive effects of nicotine and alcohol. Conditioned Place Preference (CPP) is an experimental procedure where rewarding drugs are administered in one of two distinct compartments in the CPP test apparatus. Through the process of contextual conditioning, the initially preferred compartment switches due to the association of the drugs rewarding effects with the distinct environmental features of the originally non-preferred compartment. The CPP model offers several advantages over the self-administration paradigm. For instance, specific drug effects can be examined more quickly and these effects can be viewed at precise time intervals immediately following acute or chronic exposure, both of which may not be possible with self-administration. Additionally, self-administration leads to potentially differing levels of drug exposure as it relies on the reinforcing properties of the drug to drive consumption, and reinforcement varies between animal species. There is also some evidence that the route of drug administration is important to the neurobiological effects of nicotine specifically, with subcutaneous exposure having a greater effect than the typical intravenous route associated with self-administration (Moretti et al., 2010).

CPP is detected using either nicotine or alcohol (Tzschentke, 2007; Le Foll, 2005); however, the latter is difficult to achieve in rats and depends on timing, dose,

number of conditioning trials and/or prior dependence. Importantly, few CPP studies have examined the effects of nicotine and alcohol together. Exposure to ethanol prior to nicotine conditioning enhances nicotine-induced CPP in male mice (Korkosz et al, 2006). Nicotine given before exposure to ethanol induces CPP to a greater extent than ethanol alone, but only in high locomotor rats (Philpot et al, 2014). To date no studies have examined CPP for an alcohol and nicotine drug cocktail. As both drugs are often taken together (to the point where the term 'chipping' was created to define when smoking primarily occurs while consuming alcohol), it is possible that the reward for the drug cocktail is greater than either drug alone.

One critical factor to consider when examining nicotine, which may have an important role in the interactive effects of nicotine and alcohol, is sex. Broadly, men and women smoke significantly different amounts with men smoking more than women (NHIS, 2014). Women have a higher clearance rate for nicotine and cotinine —the predominant metabolite of nicotine— after normalizing for body-weight; a result tied to the estrogen-levels of the participants (Hukkanen, Jacob, & Benowitz, 2005). Animal research replicated the difference in consumption, with male rats consuming more nicotine than female rats (Marshall et al, 2003). Importantly, previous studies found an interaction between nicotine dose and sex. Torres et al. (2009) reported that female Wistar rats have a greater CPP to moderate doses of nicotine than male Wistar rats. Torres et al. also tested low and high doses, but did not find a sex effect. Unlike the findings by Hukkanen, Jacob, and Benowitz on humans, Torres et al. found that the estrous cycle of the Wistar rats did not impact the rewarding effects of nicotine, though this may be due to low power.

Unlike nicotine, men and women vary little in percentages of those that consume alcohol. However, drinking patterns differ with men drinking at greater levels and having more negative consequences as a result (Wilsnack et al, 2000). Likewise, less robust sex differences are found in animal research, with the consumption rate of alcohol being dependent on species and dose (Witt, 2007). There is also some evidence that the sexes differ in alcohol consumption rate depending on age. Adolescent male rats consume more ethanol than their female peers, though female rats have a greater increase in alcohol consumption during puberty (Marshall et al, 2003; Witt, 2007).

Few studies have examined sex effects on the consumption of alcohol and nicotine together. In an experimental setting, Acheson (2006) found different effects of nicotine on alcohol consumption in men and women. Men had greater baseline nicotine use compared to women and had an increase in alcohol use following nicotine exposure; in contrast women decreased their alcohol consumption following nicotine exposure.

Another factor that has a significant effect on drug preference and consumption later in life is the initial stage of drug exposure. Longitudinal studies on humans who were exposed to alcohol prenatally exhibit increased use of alcohol and nicotine later in life (Pfinder et al, 2013; Yates et al, 1998; Baer et al, 2003). Animals with a history of prenatal alcohol exposure (PAE) have an increased preference to nicotine later in life (Mantella et al, 2014). Moreover, the magnitude of nicotine-induced CPP is greater in animals with a history of PAE (Roger et al, 2004). Similar results have been reported when exposure to alcohol occurs during adolescence (Baer et al, 2003).

Prenatal exposure to nicotine impacts later alcohol or nicotine use as reliably as ethanol (Baer et al, 2003), although this may be a product of limited research on nicotine

prenatal exposure. Unlike prenatal exposure, exposure to nicotine during adolescence increases alcohol consumption during adulthood in both humans and other animals (Kemppainen et al, 2009; Rinker et al, 2011). Collectively, these studies suggest that the impact of early drug exposure depends on both the type of drug and the development period during which it is consumed.

The goal of the present study was to examine the potential interactive effects of sex, PAE and adolescent drug exposure (ADE) on preference for a drug cocktail of nicotine and alcohol in adult male and female Long-Evans rats. Rats were exposed to alcohol prenatally and then alcohol, nicotine or a combination of the two drugs during adolescence. The rats were then allowed to mature into young adulthood and were tested for CPP to a cocktail of nicotine and alcohol. The moderate doses of alcohol and nicotine that were administered during adolescence and conditioning sessions were selected based on research demonstrating their ability to elicit CPP. Additionally, these moderate doses of nicotine and alcohol elicit the greatest sex-dependent effect on reward (Witt, 2007). For nicotine specifically, moderate doses produce CPP whereas lower doses that are more closely related to human cigarette use take many more trials to achieve CPP or have no effect (Matta et al., 2007).

We hypothesized that PAE would interact with ADE to affect subsequent nicotine and ethanol cocktail-induced CPP in adulthood. We speculated that drug exposure during critical developmental periods (i.e., prenatal and adolescence) would increase preference for the cocktail during adulthood with animals that were exposed during both periods having the greatest preference. In line with previous studies which have shown sex differences associated with drug use in adolescence and its impact on subsequent adult

drug use, we further hypothesized that male and female rats would differ in their preference for the cocktail with females showing a greater reward for the moderate dose of both drugs.

Methods

Animals and Treatment

The Institutional Animal Care and Use Committee at the University of New Mexico approved all experimental procedures. Husbandry and experimentation adhered to the 'Guide for the Care and Use of Laboratory Animals' (Institute of Laboratory Animal Resources on Life Sciences, National Research Council, 2011). This study consisted of two cohorts of Long-Evan rats that were run at different time points. Each cohort consisted of 80 rats, split into 2 smaller groups of 40. Each smaller group was bred 2 weeks apart to accommodate the size of the facility. The first cohort began breeding in October 2015 and the second cohort began breeding in June 2016.

Voluntary Drinking Prenatal Alcohol Exposure Paradigm:

Four-month-old Long-Evan rat breeders (Harlan Industries, Indianapolis, IN) were single-housed in plastic cages at 22 ºC and kept on a "reverse" 12-hour dark/12-hour light schedule (lights on from 2100 to 0900 hours). The rats were provided with Harlan 2920 irradiated rodent diet chow and tap water *ad libitum*. Prior to ethanol exposure there was a one-week acclimation period to the facility, during which time the rats were not handled. Following acclimation, all female rats were provided 0.066% saccharin in tap water for a four-hour period from 1000 to 1400. For the first two days the saccharin water contained 0% ethanol. This was increased to 2.5% ethanol (v/v) for two days. After the first four days, and for the rest of the exposure schedule, the saccharin water contained 5% ethanol. The daily four-hour ethanol consumption period was monitored for two weeks and the mean daily ethanol consumption for each day was recorded. At the

end of the two weeks of ethanol consumption, females that drank less than one standard deviation below the mean of all female rats were removed from the study. The remaining females were assigned to either a saccharin control (SAC) or 5% ethanol drinking group matched for mean pre-pregnancy ethanol consumption. Females were then placed with proven male breeders until pregnant as evidenced by the presence of a vaginal plug. Female rats did not consume ethanol during the breeding procedure. Beginning on Day 1 of gestation, rat dams were provided saccharin water containing either 0% or 5% ethanol for four hours a day. The volume of 0% ethanol saccharin water provided to the controls was matched to the mean volume of saccharin water consumed by the ethanol-drinking group. Daily four-hour ethanol consumption was recorded for each dam. The offspring were weighed at birth and culled to ten per litter. At PND 24 the offspring were moved into cages of two males or three females until their transfer to the permanent housing room.

Adolescent Drug Exposure Paradigm:

The rats were transported from the breeding facility to the housing facility at PND 25 to 28, depending on date of birth. The housing room maintained the same access to food and water as well as the same light cycle as the breeding room. After transportation, all rats were earmarked and placed in pair-housed cages. Cages consisted of pairings of the same sex and adolescent drug condition. Earmarking ensured that the adolescent and adult exposure paradigms were run blind to PAE condition. The rats were allowed to acclimate to the housing room without any handling for three days.

Adolescent drug exposure began on PND 31 and lasted until PND 40 for all rats. Exposure took place in a separate room, lit only by a red light. Rats were transported in their housing cages that were covered with a dark sheet. For the first cohort, exposure for females took place from 1000-1300 followed by males from 1300-1600; the order was reversed for the second cohort.

There were four ADE conditions all of which received 2 injections per day. All injections were administered using a 30-gauge needle. Ethanol injections (0.75 g/kg/ml; 20% v/v) were administered intraperitoneally (i.p.) and nicotine injections (0.6 mg/kg/ml) were administered subcutaneously (s.c.). Animals treated with the cocktail received both drug injections. A mock injection was used for injections not needing a drug dose; the control condition (saline) received two mock injections. The side of the body injected alternated every day.

Adult Condition Place Preference Paradigm

Apparatus

CPP took place in a separate testing room lit by a red light. Two separate CPP chambers were located in the testing room on a table in the middle of the room. Each apparatus consisted of two distinct compartments. One compartment was white with a grid metal floor and had corncob bedding located 2 inches below the grid floor. The second compartment was black with a bar metal floor and had pine bark bedding located 2 inches below the bar floor. A wall with a closable opening separated the two compartments. A white noise generator was located on the floor between the two compartments to simulate the noise of the housing room and reduce any extraneous noise.

A downward facing camera was located above each apparatus. The compartments were cleaned of obvious fecal matter or other detritus after every rat was run. The compartments were wiped down with 5% ethanol every time the sexes were switched. The rats were always run in the same order to acclimate rats to the smell of the rats previously run.

CPP

CPP began on PND 58 to 62, depending on date of birth. CPP was conducted over 6 consecutive days consisting of the following: one travel day, one habituation day, three conditioning days, and one final expression test day. For every session, rats were transported individually to the testing room in covered transfer cages. Rats were returned to the housing room immediately following their session and the next rats were transferred to the testing room. For the first cohort, all female rats were run first followed by the males; this was reversed for the second cohort.

Travel

Each travel day began at 1000. For the travel day, the opening between the compartments in each apparatus was open to allow free access to each compartment. The rats were placed in alternating compartments, one per apparatus, with their heads facing the far wall. They were allowed to move freely and uninterrupted in the apparatus for 10 minutes. The cameras were not recording, but the recording system was on to ensure light and sound were consistent between sessions.

Habituation

Setup for the habituation session was similar to the travel day, except the cameras recorded the time spent in each compartment. The rats were placed in the opposite

compartment they were placed in during the travel day. The time each rat spent in a compartment was used to establish a baseline compartment preference for the subsequent conditioning days. As compartment preference was needed the following day, the video was streamed to another room where a lab assistant calculated the time spent in each compartment using a stopwatch. The video was analyzed at the end of the experiment to affirm the hand-calculated times.

Conditioning

Each conditioning day began at 0900. The opening between each compartment was closed, to limit the rat's access to only the compartment they were placed in following their assigned injection. As each apparatus has two compartments and there were two test chambers, 4 rats were conditioned at the same time when appropriate. For conditioning trials, every rat received a cocktail of nicotine and ethanol at the doses given during adolescence (nicotine 0.6 mg/kg/ml and ethanol 0.75 g/kg/ml). Similar to the adolescent paradigm, rats were weighed at the end of the day and this weight was used to calculate the appropriate dose for the subsequent day; the first weight was taken at the end of the habituation day. During every session the rats received either the drug cocktail or an identical dose of saline. All nicotine injections, or nicotine equivalent saline injections, were given s.c. with 30 gauge needles. All ethanol injections, or ethanol equivalent saline injections, were given i.p. with 26 gauge needles. An increased needle size was needed to effectively administer the dose. Two sessions were run for each rat every conditioning day. For one session, the rat received the drug cocktail prior to being placed in their originally non-preferred side. For the other session, the rat received saline equivalent injections and was placed in their originally preferred side. The order of the

sessions was dependent on side preference and was alternated every day. Every rat had at least four hours between each session. The side of the body injected was alternated every session.

Preference Expression Test

The conditions for the testing session were identical to the travel day, except the cameras were recording. The time spent in each compartment during the 10-minute test session was used to get a final compartment preference.

Analysis

Video

The cameras used were part of the Lorex 1080p security camera system (model LHV828 system). The videos were converted using WinX HD Video Converter (WinX HD Converter Deluxe; Digiarty Software) and analyzed using AnyMaze software (Stoelting Co.; Wood Dale, IL, USA).

Data Analysis

All analyses were conducted using IBM SPSS Statistics for Windows, Version 22.0 (SPSS Corp., Armonk, NY). Data for each condition—the time spent in each compartment during habituation and testing sessions (seconds), the total distance traveled during the session (cm) and the distance traveled in each specific compartment (cm), distance traveled (cm) during the cocktail conditioning sessions, and the entries made into each compartment—were compared with a three-way analysis of variance (ANOVA: Sex x PAE x adolescent drug condition). These measures were calculated and analyzed using both simple final measures found on the test session as well as difference scores calculated by subtracting the respective measures of the habituation session from the test session.

Lower order ANOVAs and Scheffé test were run post hoc. As the experiment is an attempt to fill a gap in research, the Scheffé test was chosen to accommodate the potential need to compare unforeseen effects. A p-value over 0.05 (two-tailed) was considered non-significant. Interactions at $P < 0.10$ (two-tailed) were further examined even though they did not reach the significance criterion.

Results

Animal Eliminations

Four rats failed to survive until the end of the experiment. Two of these rats died from self-mutilation or cage-mate aggression, and their cage-mates were subsequently sacrificed because of the housing stipulations of the experiment. Three rats were eliminated by video recording error resulting from the Lorex system. For conditioning session analysis, further animals had to be eliminated resulting from the Lorex system corruption.

Habituation Session

Analysis of the time spent in the originally non-preferred drug-paired side (DPS) during the habituation session revealed that there were no main effects or interactions (Table 1); Sex [$F(1,149)= 1.936$, $p=0.166$], PAE [$F(1,149)= 1.159$, $p= 0.284$], ADE [$F(3,147)= 1.416$, $p= 0.241$], all interactions $p> 0.573$.

Analysis of the total distance traveled during the habituation session (Table 3) resulted in a main effect of Sex with males travelling a greater average distance than females [$F(1,149)= 10.863$, $p= 0.001$; males: 4513 cm, SD= 911 cm; females: 4042 cm, SD= 767 cm], and a main effect of PAE with PAE animals travelling a greater total distance than non-PAE animals [$F(1,149)= 4.961$, $p= 0.028$; PAE: 4450 cm, SD= 959 cm; SAC: 4135 cm, SD= 763 cm]. There was no main effect of ADE [$F(3,147)= 1.556$, $p= 0.203$] or interactions (all $p>0.128$).

Conditioning Sessions

A repeated measures ANOVA (Sex x PAE x ADE) was used to examine total distance traveled during the three conditioning sessions when the cocktail was

administered. This revealed a trend towards significance of Sex (Table 6, Figure 1) with the females travelling a greater distance across sessions [$F(1,94)= 2.921$, $p= 0.091$], and a main effect of ADE (Table 5, Figure 2) with the saline condition resulting in significantly less distance traveled across sessions than the nicotine-only condition and a marginally significant less distance traveled across sessions than the cocktail condition [$F(3,92)= 4.814$, $p= 0.004$; NIC: $p= 0.001$; COCKTAIL: $p= 0.070$]. There was also a significant interaction between PAE and ADE [$F(3,96)= 2.858$, $p= 0.041$]; PAE did not reach significance on its own [$F(1,96)= 0.608$, $p= 0.438$].

Analyses were done to further examine group differences on specific cocktail sessions (Table 5). There was a main effect of ADE on the distance traveled during the first cocktail session [$F(3,117)= 5.031$, $p= 0.003$]. Further analysis revealed that the saline condition and the nicotine-only condition differed significantly (Saline: 904 cm, SD= 653 cm; NIC: 1477 cm, SD= 791 cm; $p= 0.017$), while the saline condition showed a trend towards significance for the cocktail condition (COCKTAIL: 1373 cm, SD= 616, $p= 0.072$) and the nicotine-only condition also showed a trend towards significance being different from the ethanol-only condition (ETOH: 1021 cm, SD= 668 cm, $p= 0.093$).

Analysis of the distance traveled during the second cocktail conditioning session resulted in an interaction of PAE and ADE [$F(3,117)= 3.504$, $p= 0.018$]. Further analysis revealed that there were no significant effects from PAE across ADE groups, though ethanol-only (SAC: 1477 cm, SD= 726 cm; PAE: 2031 cm, SD= 665 cm) and cocktail (SAC: 2260 cm, SD= 1168 cm; PAE: 1872 cm, SD= 555 cm) showed a trend towards significance ($p= 0.070$ and $p= 0.071$, respectively). The above results continued when the distance traveled during the second session was covaried with the first session; PAE x

ADE [F(4,116)= 3.134, *p*= 0.029]. Collapsing across PAE groups resulted in significant differences between the saline group and nicotine-only and cocktail groups (*p*= 0.004 and *p*= 0.047, respectively). There was also a marginal difference between the nicotine-only and ethanol-only groups (*p*= 0.057).

The ANOVA of the distance traveled during the third cocktail conditioning session resulted in no main effects or interactions; Sex [F(1,119)= 1.168, *p*= 0.283], PAE [F(1,119)= 0.002, *p*= 0.961], ADE [F(3,117)= 1.315, *p*= 0.251], all interactions *p*> 0.157. Analysis of the difference between the distance traveled during the third cocktail session and the first cocktail session resulted in no main effects or interactions; Sex [F(1,119)= 0.000, *p*= 0.997], PAE [F(1,119)= 0.345, *p*= 0.558], ADE [F(3,117)= 0.802, *p*= 0.495], all interactions *p*> 0.118.

Expression Test Session

Analysis of the time spent in the DPS during the expression test session revealed there were no main effects or interactions (Table 7, Figure 3); Sex [F(1,149)= .301, *p*= 0.584], PAE [F(1,149)= .031, *p*= 0.718], ADE [F(3,147)= 1.219, *p*= 0.305], all interactions *p*> 0.305.

A main effect of Sex was found for the distance traveled in the DPS during the test session (Table 8, Figure 5) [F(1,149)= 5.800, *p*= 0.017; males: 2094 cm, SD= 721 cm; females: 1831 cm, SD= 606cm], with males covering more distance compared to the females. There were no further main effects or interactions; PAE[F(1,149)= 0.392, *p*= 0.532], ADE [F(3,147)=0.272, *p*= 0.845], all interactions *p*> 0.204. The ANOVA of the total distance traveled during the expression test (Table 10) revealed an interaction of Sex x PAE x ADE [F(3, 147)= 2.912, *p*= 0.037]; this interaction was driven by the nicotine

only adolescent drug group. Only males reached significance in this condition with the males that were exposed to alcohol prenatally and received nicotine during adolescence traveling a greater total distance than those that were not exposed to alcohol prenatally and received nicotine during adolescence [F(1,19)= 5.316, *p*= 0.033; NIC PAE: 5492 cm, SD= 1129 cm; NIC SAC: 4312 cm, SD= 1159 cm]. Females failed to reach significance but exhibited an opposite pattern compared to males, with the females that were exposed to alcohol prenatally and received nicotine during adolescence traveling less total distance than those that were not exposed to alcohol prenatally and received nicotine during adolescence [F(1,16)= 1.664, *p*= 0.217; NIC PAE: 2880 cm, SD= 2086 cm; NIC SAC: 3749 cm, SD= 462 cm]. No further significant interactions were detected between conditions.

The assessment of the total entries into the DPS during the test session (Table 9) revealed a marginal effect of Sex with males averaging more entries compared to the females [F(1,149)= 3.876, *p*= 0.051; males: 26.9, SD= 10.4; females: 23.8, SD= 8.0]. There were no further main effects or interactions; PAE [F(1,149)= 0.268, *p*= 0.606], ADE [F(3,147)=1.288, *p*= 0.281], all interactions *p*> 0.169.

Test Session Covaried with Baseline

Analysis of the time spent in the DPS during the test session covaried with the time spent in the compartment during habituation indicated there were no interactions or main effects; Sex [F(1,148)= 0.477, *p*=0.491], PAE [F(1,148)= .062, *p*= 0.804], ADE [F(3,146)= 1.243, *p*= 0.297], all interactions *p*> 0.305.

The ANOVA of the distance traveled in the DPS during test session covaried with the time spent in the non-preferred compartment during the habituation session indicated

there was a main effect of Sex with males traveling more distance compared to females [F(1,148)= 6.438, *p*= 0.012]. There were no further main effects or interactions; PAE [F(1,148)= 0.002, *p*= 0.968], ADE [F(3,146)=0.268, *p*= 0.848], all interactions *p*> 0.165. The analysis of the total distance traveled during the test session covaried by the total distance traveled during the habituation session resulted in an interaction of Sex x PAE x ADE [F(3, 146)= 3.182, *p*= 0.026]. For the females, there were no significant differences between the adolescent groups regardless of PAE. Similarly, for the males there were no significant differences between the adolescent groups regardless of PAE. However, the distance traveled by the adolescent exposure saline and nicotine only groups was greater for the PAE rats whereas non-PAE rats traveled a greater distance for the other adolescent exposure conditions.

The analysis of the entries in the DPS during the test session covaried with the entries in the chamber during the habituation session indicated there were no interactions or main effects; Sex [F(1,148)= 1.434, *p*= 0.233], PAE [F(1,148)= 0.327, *p*= 0.568], ADE [F(3,146)= 0.971, *p*= 0.409], all interactions *p*> 0.163.

Difference Scores (Test Session – Habituation Session)

Analysis of the difference in time spent in the DPS revealed no interactions or main effects (Table 11, Figure 4); Sex [F(1,149)= .044, *p*= 0.835], PAE [F(1,149)= 0.671], *p*= 0.414], ADE [F(3,147)= 1.151, *p*= 0.331], all interactions *p*> 0.662.

A main effect of Sex was detected for the difference in distance traveled in the DPS (Table 12) with males averaging a greater distance traveled during the test session than the habituation session, while the females averaged a reduction in distance traveled [F(1,149)= 5.316, *p*= 0.023; males: 166 cm, SD= 636; females: -67.4 cm, SD= 702.6].

There were no further main effects or interactions; PAE [F(1,149)= 0.548, *p*= 0.461], ADE [F(3,147)=0.375, *p*= 0.772], all interactions *p*> 0.182. The ANOVA of the difference in total distance traveled (Table 13) detected no main effects or interactions; Sex [F(1,149)= 2.591, *p*=0.110], PAE [F(1,149)= 0.019, *p*= 0.891], ADE [F(3,147)= 1.109, *p*= 0.348], all interactions *p*> 0.642.

There were no main effects or interactions for the difference in entries in the DPS; Sex [F(1,149)= .004, *p*= 0.949], PAE [F(1,149)= 0.319, *p*= 0.573], ADE [F(3,147)= 0.503, *p*= 0.681], all interactions *p*> 0.239.

Percent Change

The percent change of the time spent in the DPS resulted in no main effects or interactions (Table 14); Sex [F(1,148)= 0.187, *p*= 0.666], PAE [F(1,148)= 1.334, *p*= 0.250], ADE [F(3,146)= 0.744, *p*= 0.528], all interactions *p*> 0.745.

Discussion

Contrary to our prediction, we did not detect an interaction between PAE and ADE on reward for a cocktail of nicotine and alcohol during adulthood. Indeed, there were no significant group differences in the total time spent in DPS or the change in time spent in the DPS (Tables 7 and 11, Figures 3 and 4). These results indicate that either the drug cocktail failed to illicit a significant reward in any of the groups, or our CPP test was not sensitive enough to identify the reward for the drug cocktail. The high societal rates of nicotine and alcohol co-use support the idea that the drugs are rewarding taken together, and therefore it is more likely that our null effects result from having too low of sensitivity to produce CPP for the drug cocktail.

We chose to utilize an unbiased apparatus rather than a biased apparatus to examine a cocktail of alcohol and nicotine CPP, as this procedure is more sensitive to the rewarding effects of a drug cocktail (Tzschentke, 2007). Our results indicate that our apparatus was unbiased, as the baseline preference for the black or white compartments during the habituation session was not different between any of the group conditions (Table 1). Furthermore, by pairing the drug cocktail with the rat's initially non-preferred side (biased design) we maximized our ability to detect a change in preference/drug reward (Tzschentke, 2007). Therefore our inability to detect a preference change (i.e., CPP) was not likely the result of our experimental design (e.g., biased vs. unbiased design).

Surprisingly the PAE animals showed increased locomotion during the habituation session (Table 3). PAE exposure is associated with hyperactivity, though the

moderate doses we used have not been consistent in eliciting hyperactivity in later life. In fact, different doses, exposure times, and species of animal have resulted in opposite patterns of locomotion (Marquardt & Brigman, 2016). It may also be that the increased locomotion is a result of a decreased anxiogenic-like state; though again this has not been a robust result using moderate doses and varies greatly depending on PAE methodology (Staples et al., 2013; Marquardt & Brigman, 2016). Our travel day was designed to get the animals acquainted with the compartments and thus reduce anxiety during the habituation session, though the 10 minutes may not have been long enough to reduce anxiety. It is also interesting that hyperactivity was found in both sexes as previous research has only shown differences in male behavioral differences following moderate PAE (Rodriguez et al., 2016; Marquardt & Brigman, 2016).

There are several potential shortcomings that could be addressed in future studies examining nicotine and ethanol-induced CPP. In the current experiment, the CPP test occurred weeks after ADE and the duration of ADE was only ten days. The adolescent exposure period could be increased or occur closer in time to the CPP test so that the animals are experiencing withdrawal during the conditioning sessions. These have been shown to increase drug reward and thus increase the ability for the CPP paradigm to identify the conditional differences (Tzschentke, 2007). Raising and housing animals in low stimuli-enriched environments or by limiting food access before testing sessions can also increase drug reward. The inclusion of four different ADE conditions required a large number of animals, thus we choose to use one moderate dose of nicotine and ethanol throughout the experiment. It may be that PAE and ADE differences in reward would manifest at different drug doses. Different doses during prenatal and adolescent

exposures may also change the tolerance or the reward for the cocktail as adults. As humans vary in their smoking and drinking amounts, future studies will need to examine different drug doses. More drug conditioning sessions could increase the rat's ability to associate the non-preferred compartment with the drug. Although more conditioning sessions do not always increase the CPP paradigm's sensitivity to detect reward. For example, Torres et al. (2009) performed alcohol-conditioning sessions on female rats for 30 days and found no evidence of reward. Researchers need to be mindful when designing CPP experiments as the research question being answered may change with the changes to the methodology.

It is difficult to achieve CPP with alcohol, however methods have been manipulated to increase sensitivity to detect alcohol reward and/or increase the rewarding effects of alcohol itself (Tzschentke, 2007). Age appears to be an important factor with trends towards significant differences in reward disappearing by adulthood (Philpot, Badanich, & Kirstein, 2003). Alcohol doses are often limited to low to moderate (in this study we used moderate) as higher doses begin to show conditioned place aversion instead of preference, though the low to moderate doses are not robust in producing preference. Extensive research has shown PAE is closely linked to increased alcohol consumption in later life, but the reward of alcohol hasn't been studied to the same degree (Tzschentke, 2007; Ceccanti et al., 2015). PAE has been shown to increase subsequent reward to alcohol but this is limited to neonatal or adolescent exposure and the increase often only exhibits a trend towards significance (Chotro, Arias, & Laviola, 2007; Ceccanti et al., 2015). Barbier et al. (2009) did find increased preference to the reward of ethanol following PAE, but this was only seen in male rats. It is thus not unique that our

findings did not detect a preference to the reward of alcohol as we studied preference in adults, though we believed the addition of nicotine to the cocktail and the extensive drug-exposure histories would have led to preference developing to the moderate doses.

Although we did not detect any differences for any of the test session duration measures, our results do indicate a difference in locomotion resulting from the conditioning sessions. The analysis of the total distance traveled during the test session revealed an interaction indicating that the males exposed to only nicotine during adolescence had an increase in total distance traveled if they were also exposed to alcohol prenatally compared to non-PAE rats (Table 10). The overall interaction remained when the total distance traveled during the test session was covaried with the total distance traveled during the habituation session. The increase in general locomotion from exposure to the cocktail (as demonstrated by the result remaining after covarying locomotion during the habituation session) could occur due to several variables. It may be that the rats were exhibiting increased exploration for the cocktail (i.e., drug-seeking behavior) and that the specific compartment associations were not successfully conditioned (i.e., the rats did not associate the initially non-preferred compartment with the cocktail). Alternatively, there could be different anxiolytic-like effects of the cocktail that led to more general exploration, not necessarily related to the compartment cocktail association/conditioning.

The significant differences in the non-preferred compartment measures enhance our ability to interpret the results. The distance traveled in only DPS during the test session revealed a main effect of sex, with on average males traveling more distance than females (Table 8, Figure 5). The main effect of sex remained when the distance traveled

in the DPS during the habituation session was covaried with the test session. This indicates that it was not simply the difference between the sexes in baseline locomotion that resulted in the test session difference, but rather a lasting effect of exposure to the drug cocktail. The measure of entries into the non-preferred compartment during the test session (Table 9) supported the sex differences, with males having more entries than females. The compartment-specific difference in locomotion may indicate an increase in exploration for the cocktail (drug-seeking behavior), or as a decrease in the anxiogenic-like state associated with the compartment. It should be noted that these are not mutually exclusive outcomes. Regardless of the reason for the increase in compartment-specific locomotion, the increase is indirect evidence of preference for the cocktail for only the male rats.

In addition to the distance measures during habituation and test sessions, we analyzed the distance traveled during the conditioning sessions when the drug cocktail was onboard (Table 5, Figure 2). This served as an index of whether the rats were developing a tolerance or sensitization for the locomotor effects of the drugs across the three cocktail-conditioning sessions. When administered for the first time, the cocktail produced the lowest distance scores across the three sessions. As the cocktail was repeatedly administered, the distance scores increased to the level of the saccharine conditioning sessions. This illustrates tolerance developing to the cocktail's suppressing effect on locomotion or the beginning of sensitization (Table 4). Overall, we found that this pattern was dependent on previous adolescent nicotine exposure and that females responded had a trend towards greater levels of locomotion following cocktail exposure than males.

Further analysis was conducted to examine how the groups varied at each drug conditioning session. Results from the first drug session revealed that the difference in total distance traveled was dependent on adolescent exposure to nicotine, with the nicotine-only and cocktail conditions having a greater total distance traveled compared to the saline and ethanol-only conditions (Table 5, Figure 2). These group differences were present regardless of sex or PAE conditions. As prior exposure to nicotine led to the greatest total distance traveled in the first session and across later sessions, it could be inferred that nicotine serves as an influential moderator of the negative effects of alcohol as it led to the greatest baseline tolerance to the cocktail. As the ethanol-only condition did not result in similar levels of tolerance to the cocktail, it suggests a less influential role for ethanol as a moderator of the negative effects of the cocktail, at least for the intimal exposures.

As opposed to the distance measures of the first session that were primarily driven by adolescent exposure to nicotine, the ADE conditions interacted with PAE to differentially impact distance measures during the second drug session (Table 5, Figure 2). The PAE exposure resulted in a trend towards significance of differences in total distance for the ethanol-only and cocktail adolescent exposure conditions. These two conditions had opposing overall distance patterns, with PAE resulting in a greater total distance in the ethanol-only ADE condition, while resulting in less total distance in the cocktail condition. As neither condition in which the drugs were given alone resulted in the locomotor pattern of the cocktail group, it is probable that this is the result of an interaction of exposure to the two drugs during adolescence. It is also worth noting that the different PAE pattern for distance is true across all groups and all sessions, but it only

reaches significance in difference from the other groups for session two. This could mean that the rats that were exposed to alcohol prenatally and had the cocktail as adolescents could have a lower tolerance to the cocktail as adults compared to those than only had the cocktail exposure during adolescence. For all other adolescent exposure conditions PAE resulted in a similar or greater tolerance to the cocktail as adults.

Collapsing across PAE conditions in the second drug session resulted in significant differences between the saline condition and the nicotine-only and cocktail conditions (Table 5). The nicotine-only and ethanol-only groups also showed a trend of difference in locomotion. This again suggests nicotine's role as a moderator of the cocktail, for any previous exposure to nicotine led to increased tolerance to the cocktail. By the third drug session, no significant differences in distance traveled were present for any previous drug exposure or sex condition, suggesting that all groups developed tolerance to the locomotor suppressing effects of the drug cocktail.

The female rats had a greater natural tolerance to the cocktail than the males, as evident by their trend towards greater distance scores across the drug conditioning sessions (Table 6, Figure 1). This is not surprising as males and females react differently to the locomotor effects of nicotine (Hukkanen, Jacob, & Benowitz, 2005; Marshall et al, 2003). It is surprising that we only found indirect evidence of reward for the cocktail for the male rats, as moderate nicotine doses like those used in the study tend to show a greater reward for female rats (Torres et al., 2009). The greater cocktail tolerance in the female rats may explain why only the males showed indirect evidence of reward. It is possible the sex-dependent, moderating effects of nicotine also negated some of the positive effects of the cocktail as well, which led to a less rewarding experience.

We did not detect any differences in reward due to previous PAE, though this does not mean the groups did not differ. It is possible that memory deficits in the PAE group lowered the association strength of the cocktail to the compartment and thus wiped out potential differences in reward. PAE has been found to result in robust deficits in hippocampal-dependent learning, a process involved in developing the drug-context paired association needed for the expression of CPP (Marquardt & Brigman, 2016; Savage et al., 1989; Morrisett et al., 1989; Reyes, Wolfe, & Savage, 1989). Future studies using PAE and the CPP paradigm should first examine a drug (e.g., cocaine) known to produce a strong association to see to what degree, if any, the PAE impacted association ability.

Our findings suggest exposure to the two drugs in immediate succession may not be the most applicable model for human use. It may be that the high societal rates of nicotine and alcohol co-use come from regulatory effects of taking the two drugs together, as opposed to a direct increase in reward. People often smoke while drinking, having the subsequent effect of increasing the total amount they drank during the sitting (Barrett et al, 2006; Kouri et al, 2004). The increase in drinking could be the result of nicotine either increasing the positive effects of alcohol, or decreasing the negative effects of alcohol. Our female-specific increase in tolerance did not result in a female-specific increase in reward. This suggests that it is nicotine moderating the negative, nauseating effects of alcohol that in turn allows one to drink more in a sitting. Previous research provides further evidence for nicotine's specific moderating role as exposure to nicotine before alcohol exposure increases alcohol consumption while alcohol pre-exposure does not increase nicotine consumption (Guavin et al, 1993; Boutros et al, 2015; Mantella et al

2014; Darbra et al, 2004). If we allowed the rats to self-administer the cocktail, we may have been able to detect differences in reward by letting the rats consume a greater amount of the cocktail.

The mechanisms of how nicotine and alcohol interact remain mostly unknown, yet there is an increasing amount of evidence indicating a similar effect on neuronal pathways (Funk et al, 2006). The mesolimbic dopamine (DA) system, an area of the brain associated with incentive-based behavior, has been identified as an important area that modulates rewarding effects of drug exposure. Both nicotine and alcohol increase DA release in the mesolimbic system and have been shown to release a greater amount of DA when given together (Tizabi et al., 2002). Nicotine and alcohol increase DA release indirectly through the excitation of nicotinic acetylcholine receptors (nAChR) (Doyon et al., 2013, Tizabi et al., 2002). Future studies should examine this effect, as DA release in the mesolimbic system is associated with drug reward and addiction. Examining DA and nAChR levels during our CPP paradigm may have revealed more about the how the rats differed in locomotion and reward to the cocktail.

Deviations across all time and distance scores were large, regardless of sex or previous drug exposure condition. This may have resulted from a variable that we did not measure, which had a large influence on reward to the cocktail. Previous studies found inherent characteristics, which can vary largely within rat species, can have significant effects on alcohol drinking and other drug taking behaviors. Characteristics such as sensation seeking have been manipulated to modulate inherent drug reward and have even allowed the CPP paradigm to better distinguish drug reward when it was previously unable to do so (Tzschentke, 2007; Philpot et al, 2014). However, selecting rat species

specifically for characteristics that affect drinking amounts (such as using only high-locomotor rats) changes the research question being asked and answered by the experiment.

In summary, we found that there were significant differences between the sexes in how they reacted to exposure to the cocktail as adults. We found that the females had a moderately greater tolerance to the cocktail than the males, though males showed indirect evidence of having reward for the cocktail when the females did not. In addition to the sex effects, we found that the animals that were exposed to nicotine during adolescence also had a greater tolerance to the cocktail than those that were not. It was also found that PAE resulted in a greater tolerance to the cocktail for all adolescent conditions, except for when the cocktail was also given as adolescents. Our findings suggest a moderating role for nicotine when it is consumed with alcohol, which specifically lessens the negative effects of consuming alcohol. Future studies should examine and expand upon nicotine's possible moderating role, as it may be what is leading to the high rates of societal use.

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Tables & Figures

Table 1 Habituation Session Drug-Paired Compartment Times

Adolescent Group		Saline (*36*)	NIC (*37*)	ETOH (*37*)	Cocktail (*39*)	Average	
Male (*77*)	SAC (*37*)	249 (47)	255 (26)	260 (51)	239 (39)	251 (41)	253 (36)
	PAE (*40*)	253 (37)	262 (17)	262 (28)	247 (41)	257 (31)	
Female (*74*)	SAC (*34*)	264 (39)	257 (41)	251 (32)	260 (28)	258 (34)	260 (34)
	PAE (*38*)	267 (28)	278 (20)	272 (4)	245 (32)	263 (33)	
Combined	SAC	256 (43)	256 (34)	256 (42)	250 (35)	254 (38)	
	PAE	259 (33)	269 (19)	267 (34)	246 (35)	260 (32)	
		258 (38)	262 (28)	261 (38)	248 (35)		257 (36)

Italicized numbers in brackets indicate group number of rats included in the analysis. All times are in seconds with standard deviation following in brackets.

Table 2 Habituation Session Drug-Paired Compartment Distance Traveled

Adolescent Group		Saline (*36*)	NIC (*37*)	ETOH (*37*)	Cocktail (*39*)	Average	
Male (*77*)	SAC (*37*)	1741 (445)	1728 (504)	1962 (449)	1951 (525)	1846 (477)	1928 (474)
	PAE (*40*)	1883 (416)	2266 (444)	2057 (569)	1833 (308)	2018 (461)	
Female (*74*)	SAC (*34*)	2033 (623)	1784 (280)	1756 (672)	1822 (299)	1839 (485)	1898 (546)
	PAE (*38*)	1944 (381)	2270 (669)	1932 (919)	1808 (406)	1964 (608)	
Combined	SAC	1870 (535)	1756 (398)[b]	1859 (566)	1887 (421)	1842 (477)[a]	
	PAE	1910 (390)	2268 (528)[b]	1998 (732)	1819 (359)	1922 (533)[a]	
		1890 (462)	1991 (524)	1923 (642)	1854 (388)		1914 (509)

Italicized numbers in brackets indicate group number of rats included in the analysis. All scores are in centimeters with standard deviation in centimeters following in brackets.

[a] Marginally significant difference ($p = 0.073$)

[b] Significant difference ($p = 0.003$)

Table 3 Habituation Session Total Distance Traveled

Adolescent Group		Saline (*36*)	NIC (*37*)	ETOH (*37*)	Cocktail (*39*)	Average	
Male (*77*)	SAC (*37*)	4270 (922)	4393 (943)	4207 (757)	4502 (587)	4343 (791)	4513 (910)[a]
	PAE (*40*)	4237 (898)	5431 (955)	4593 (1034)	4474 (770)	4697 (1002)	
Female (*74*)	SAC (*34*)	4154 (870)	3853 (514)	3874 (883)	3835 (424)	3917 (675)	4042 (767)[a]
	PAE (*38*)	4100 (476)	4496 (1032)	4070 (1259)	4122 (604)	4181 (846)	
Combined	SAC	4218 (874)	4123 (789)	4041 (818)	4168 (605)	4135 (763)[b]	
	PAE	4176 (725)	5046 (1066)	4347 (1140)	4270 (682)	4450 (960)[b]	
		4197 (792)	4547 (1025)	4181 (977)	4218 (637)		4285 (874)

Italicized numbers in brackets indicate group number of rats included in the analysis. All scores are in centimeters with standard deviation in centimeters following in brackets.

[a] Significant difference ($p < 0.001$)

[b] Significant difference ($p = 0.028$)

Table 4 Conditioning Saline Sessions Total Distance Traveled

Drug Session		One	Two	Three	One Collapsed	Two Collapsed	Three Collapsed
Control (*30*)	SAC (*17*)	2056 (562)	2000 (549)	2048 (796)	2240 (569)	1838 (526)	2013 (660)
	PAE (*13*)	2480 (501)	1626 (426)	1967 (452)			
NIC (*27*)	SAC (*16*)	2230 (642)	1942 (571)	2067 (429)	2360 (732)	2217 (771)	2184 (596)
	PAE (*11*)	2548 (843)	2617 (873)	2354 (772)			
ETOH (*28*)	SAC (*17*)	2200 (826)	1961 (653)	1924 (500)	2223 (727)	1942 (630)	2011 (611)
	PAE (*11*)	2259 (576)	1913 (621)	2146 (757)			
Cocktail (*27*)	SAC (*14*)	2284 (659)	2345 (828)	2614 (974)	2267 (717)	2220 (791)	2547 (898)
	PAE (*13*)	2249 (802)	2085 (759)	2474 (843)			

Italicized numbers in brackets indicate group number of rats included in the analysis. All scores are in centimeters with standard deviation in centimeters following in brackets.

Table 5 Conditioning Cocktail Sessions Total Distance Traveled

Drug Session		One	Two	Three	One Collapsed	Two Collapsed	Three Collapsed
Control (*32*)	SAC (*17*)	927 (637)	1472 (383)	2031 (752)	904 (653)[a,b]	1529 (608)[f,g]	1948 (676)
	PAE (*15*)	878 (693)	1594 (801)	1855 (589)			
NIC (*29*)	SAC (*17*)	1241 (621)	2070 (722)	2274 (939)	1477 (791)[a,c]	2220 (706)[f,h]	2380 (794)
	PAE (*12*)	1812 (907)	2431 (652)	2530 (531)			
ETOH (*30*)	SAC (*18*)	1014 (713)	1477 (726)[d]	1963 (679)	1021 (668)[c]	1699 (743)[h]	2062 (941)
	PAE (*12*)	1030 (626)	2031 (665)[d]	2210 (1258)			
Cocktail (*29*)	SAC (*15*)	1432 (719)	2359 (972)[e]	2260 (1167)	1374 (616)[b]	2057 (899)[g]	2073 (930)
	PAE (*14*)	1312 (501)	1733 (709)[e]	1872 (555)			

Italicized numbers in brackets indicate group number of rats included in the analysis. All scores are in centimeters with standard deviation in centimeters following in brackets.

[a, f, g] Significant difference ($p < 0.05$)

[b, c, d, e, h] Marginally significant difference ($p < 0.10$)

Table 6 Conditioning Session Distance Traveled

Conditioning Day		One	Two	Three
Male (*60*)[a]	Saline	2165 (736)	2106 (688)	2232 (766)
	Cocktail	1104 (670)	1820 (812)	2034 (821)
Female (*50*)[a]	Saline	2389 (592)	1994 (714)	2139 (677)
	Cocktail	1309 (739)	1969 (781)	2230 (917)

Italicized numbers in brackets indicate group number of rats included in the analysis. All scores are in centimeters with standard deviation in centimeters following in brackets.

[a] Marginally-significant difference ($p = 0.091$)

Table 7 Test Session Drug-Paired Compartment Times

Adolescent Group		Saline (*36*)	NIC (*37*)	ETOH (*37*)	Cocktail (*39*)	Average	
Male (*77*)	SAC (*37*)	291 (58)	310 (45)	277 (82)	273 (62)	292 (64)	285 (59)
	PAE (*40*)	267 (50)	290 (52)	292 (58)	267 (50)	289 (59)	
Female (*74*)	SAC (*34*)	298 (62)	293 (70)	267 (76)	311 (44)	288 (63)	290 (61)
	PAE (*38*)	311 (52)	305 (36)	255 (83)	288 (51)	282 (57)	
Combined	SAC	294 (58)	301 (58)	272 (77)	292 (56)	290 (63)	
	PAE	287 (54)	296 (46)	275 (71)	286 (60)	286 (58)	
		290 (55)	299 (52)	273 (73)	289 (57)		288 (60)

Italicized numbers in brackets indicate group number of rats included in the analysis. All times are in seconds with standard deviation following in brackets.

Table 8 Test Session Drug-Paired Compartment Distance Traveled

Adolescent Group		Saline (*36*)	NIC (*37*)	ETOH (*37*)	Cocktail (*39*)	Average	
Male (*77*)	SAC (*37*)	1839 (484)	1975 (697)	2074 (932)	2117 (528)	2001 (665)	2094 (721)[a]
	PAE (*40*)	2019 (709)	2721 (875)	2016 (556)	1959 (740)	2195 (773)	
Female (*74*)	SAC (*34*)	1862 (569)	1794 (311)	1763 (649)	1954 (508)	1842 (506)	1831 (606)[a]
	PAE (*38*)	1959 (525)	1610 (1172)	1743 (809)	1904 (358)	1818 (710)	
Combined	SAC	1849 (508)	1885 (534)	1919 (798)	2035 (511)	1924 (595)	
	PAE	1993 (617)	2264 (1124)	1888 (679)	1927 (533)	2015 (762)	
		1921 (562)	2059 (865)	1904 (735)	1982 (518)		1967 (679)

Italicized numbers in brackets indicate group number of rats included in the analysis. All scores are in centimeters with standard deviation in centimeters following in brackets.

[a] Significant difference ($p = 0.017$)

Table 9 Test Session Drug-Paired Compartment Entries

Adolescent Group		Saline (*36*)	NIC (*37*)	ETOH (*37*)	Cocktail (*39*)	Average	
Male (*77*)	SAC (*37*)	26.2 (6.4)	27.1 (9.2)	27.6 (12.3)	24.5 (10.0)	26.4 (9.4)	26.9 (10.4)[a]
	PAE (*40*)	22.3 (9.2)	32.9 (13.0)	25.7 (14.3)	29.0 (9.9)	27.4 (11.4)	
Female (*74*)	SAC (*34*)	20.3 (11.0)	24.9 (5.4)	23.6 (12.4)	25.6 (6.7)	23.8 (9.1)	23.8 (8.0)[a]
	PAE (*38*)	24.8 (4.0)	25.3 (5.4)	25.3 (9.4)	20.5 (6.5)	23.7 (6.7)	
Combined	SAC	23.6 (9.0)	26.0 (7.4)	25.6 (12.2)	25.1 (8.3)	25.1 (9.3)	
	PAE	23.4 (7.2)	29.9 (9.4)	25.5 (11.9)	24.1 (8.9)	25.7 (9.6)	
		23.5 (8.0)	27.8 (8.5)	25.5 (11.9)	24.6 (8.5)		25.4 (9.4)

Italicized numbers in brackets indicate group number of rats included in the analysis. All scores number of entries with standard deviation in number of entries following in brackets.

[a] Marginally significant difference ($p = 0.051$)

Table 10 Test Session Total Distance Traveled

Adolescent Group		Saline (*36*)	NIC (*37*)	ETOH (*37*)	Cocktail (*39*)	Average	
Male (*77*)	SAC (*37*)	3955 (770)	4312 (1159)[a]	4221 (667)	4359 (712)	4212 (832)	4387 (986)
	PAE (*40*)	4329 (1018)	5492 (1129)[a]	4109 (984)	4271 (762)	4577 (1110)	
Female (*74*)	SAC (*34*)	3734 (894)	3749 (462)	3772 (709)	3763 (610)	3756 (645)	3703 (942)
	PAE (*38*)	3876 (451)	2880 (2086)	3846 (1268)	3815 (588)	3644 (1199)	
Combined	SAC	3857 (810)	4030 (906)	3997 (708)	4061 (714)	3990 (776)	
	PAE	4127 (828)	4416 (2026)	3985 (1098)	4007 (687)	4130 (1237)	
		3992 (819)	4208 (1515)	3991 (895)	4035 (692)		4057 (1021)

Italicized numbers in brackets indicate group number of rats included in the analysis. All scores are in centimeters with standard deviation in centimeters following in brackets.

[a] Significant difference ($p = 0.033$)

Table 11 Drug-Paired Compartment Time Difference (Test Session – Habituation Session)

Adolescent Group		Saline (*36*)	NIC (*37*)	ETOH (*37*)	Cocktail (*39*)	Average	
Male (*77*)	SAC (*37*)	42 (80)	54 (37)	17 (103)	42 (80)	37 (76)	32 (72)
	PAE (*40*)	14 (61)	27 (51)	29 (75)	35 (91)	26 (67)	
Female (*74*)	SAC (*34*)	34 (91)	36 (81)	16 (98)	50 (54)	34 (80)	30 (73)
	PAE (*38*)	43 (69)	27 (36)	-16 (69)	43 (66)	26 (65)	
Combined	SAC	38 (83)	45 (62)	16 (98)	42 (65)	36 (78)	
	PAE	27 (65)	27 (44)	8 (74)	40 (75)	26 (66)	
		33 (73)	37 (55)	13 (87)	41 (69)		31 (72)

Italicized numbers in brackets indicate group number of rats included in the analysis. All times are in seconds with standard deviation following in brackets.

Table 12 Drug-Paired Compartment Distance Traveled Difference (Test Session – Habituation Session)

Adolescent Group		Saline (*36*)	NIC (*37*)	ETOH (*37*)	Cocktail (*39*)	Average	
Male (*77*)	SAC (*37*)	97 (520)	247 (474)	112 (994)	166 (619)	155 (659)	166 (636)[a]
	PAE (*40*)	136 (551)	455 (601)	-40 (676)	126 (649)	177 (619)	
Female (*74*)	SAC (*34*)	-171 (815)	10 (416)	7 (625)	131 (470)	3 (572)	-67 (703)[a]
	PAE (*38*)	15 (676)	111 (263)	-189 (534)	95 (463)	-146 (826)	
Combined	SAC	-22 (660)	129 (451)	60 (810)	149 (535)	81 (619)	
	PAE	82 (594)	-4 (1142)	-111 (599)	108 (532)	22 (739)	
		30 (621)	68 (831)	-19 (717)	129 (527)		53 (677)

Italicized numbers in brackets indicate group number of rats included in the analysis. All scores are in centimeters with standard deviation in centimeters following in brackets.

[a] Significant difference ($p = 0.023$)

Table 13 Total Distance Traveled Difference (Test Session – Habituation Session)

Adolescent Group		Saline (*36*)	NIC (*37*)	ETOH (*37*)	Cocktail (*39*)	Average	
Male (*77*)	SAC (*37*)	-315 (576)	-82 (582)	14.3 (775)	-143 (578)	-131 (621)	-126 (712)
	PAE (*40*)	92 (1033)	61 (975)	-484 (530)	-203 (383)	-120 (807)	
Female (*74*)	SAC (*34*)	-419 (842)	-104 (634)	-102 (841)	-71.1 (515)	-161 (698)	-339 (1018)
	PAE (*38*)	-224 (462)	-1616 (2439)	-223 (617)	-307 (538)	-537 (1268)	
Combined	SAC	-361 (686)	-93 (593)	-44 (790)	-107 (534)	-146 (655)	
	PAE	-48 (824)	-630 (1868)	-361 (570)	-263 (470)	-320 (1067)	
		-205 (764)	-339 (1345)	-189 (707)	-183 (503)		-229 (877)

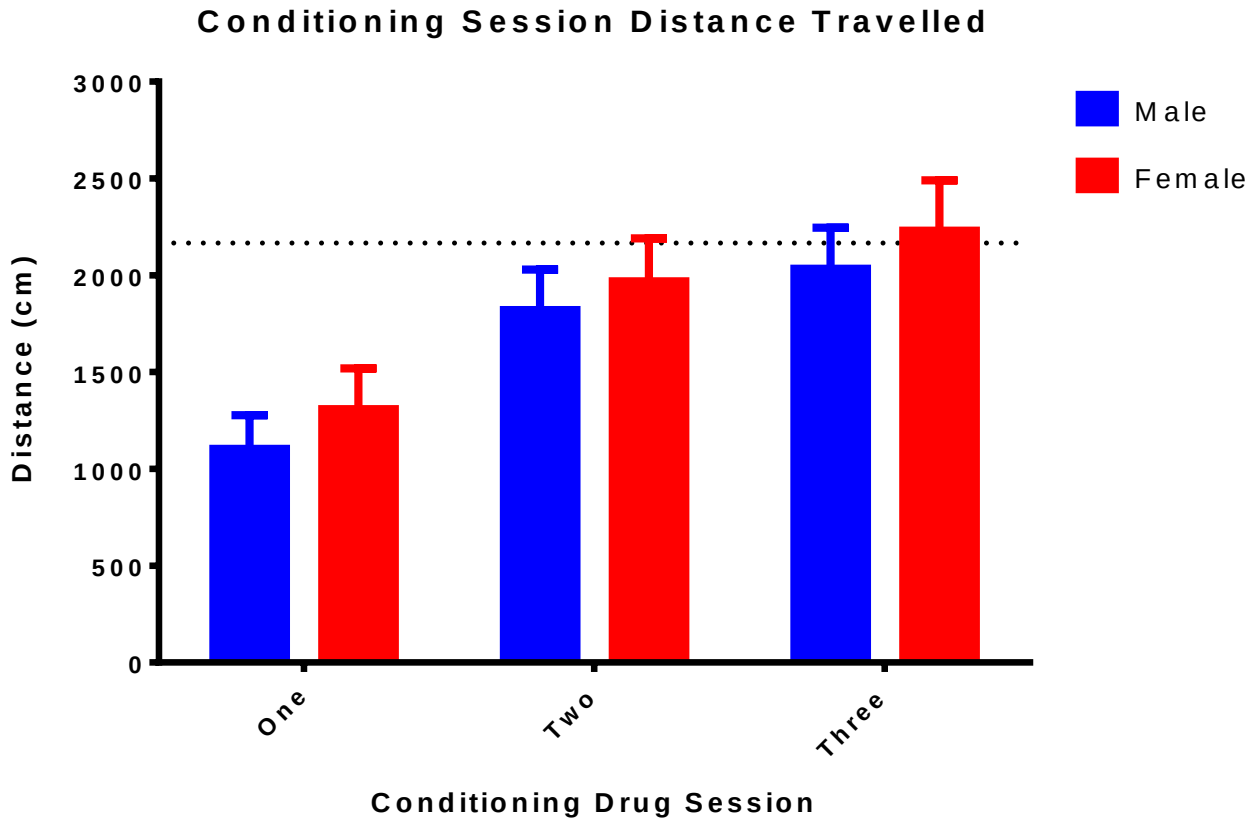
Italicized numbers in brackets indicate group number of rats included in the analysis. All scores are in centimeters with standard deviation in centimeters following in brackets.

Table 14 Time in Drug-Paired Compartment Percent Change

Adolescent Group		Saline (*36*)	NIC (*37*)	ETOH (*37*)	Cocktail (*39*)	Average	
Male (*77*)	SAC (*37*)	23 (44)	22 (15)	15 (38)	18 (38)	20 (40)	16 (32)
	PAE (*40*)	7 (24)	11 (20)	14 (36)	18 (38)	12 (29)	
Female (*74*)	SAC (*34*)	18 (42)	17 (36)	10 (42)	21 (24)	17 (35)	14 (32)
	PAE (*38*)	18 (28)	10 (13)	-7 (26)	20 (32)	11 (28)	
Combined	SAC	21 (42)	19 (27)	13 (49)	20 (31)	18 (37)	
	PAE	12 (26)	10 (17)	4 (32)	19 (33)	12 (28)	
		17 (35)	15 (23)	9 (42)	20 (32)		15 (33)

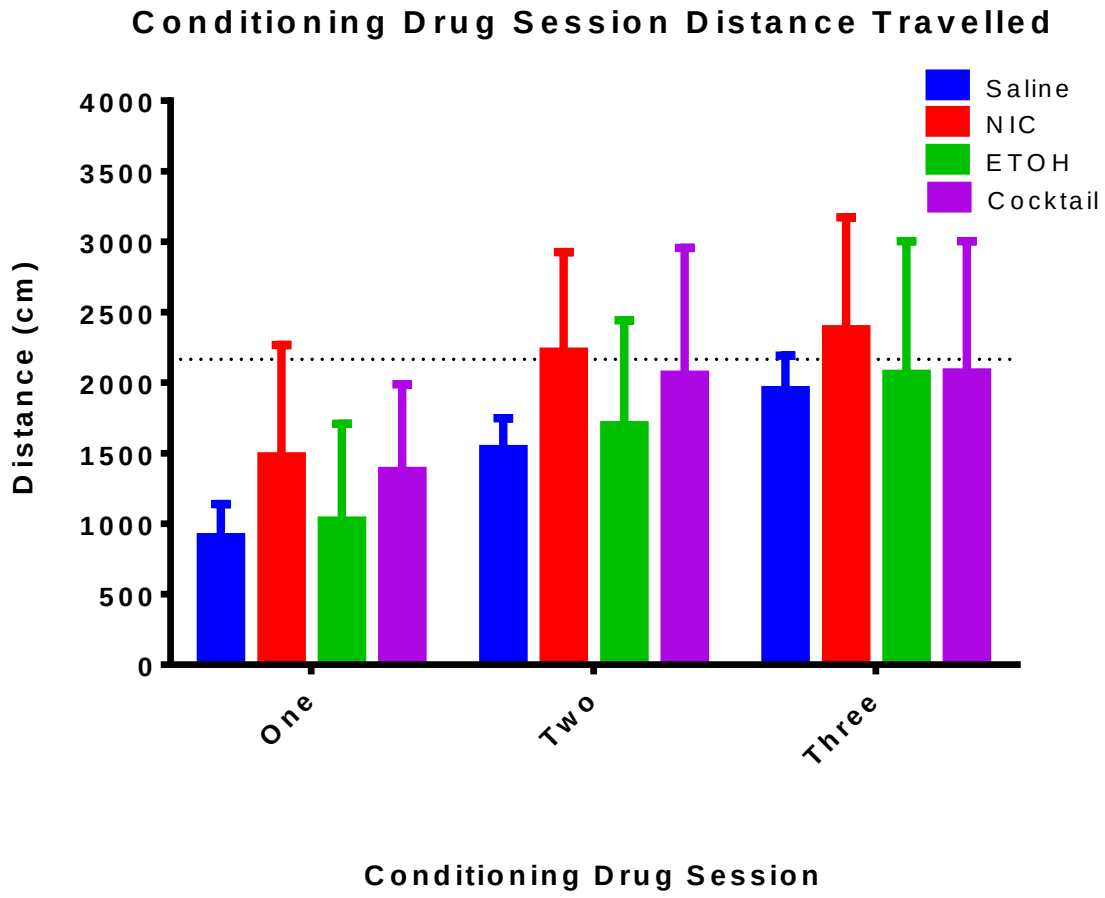
Italicized numbers in brackets indicate group number of rats included in the analysis. All scores are in seconds with standard deviation in seconds following in brackets.

Figure 1



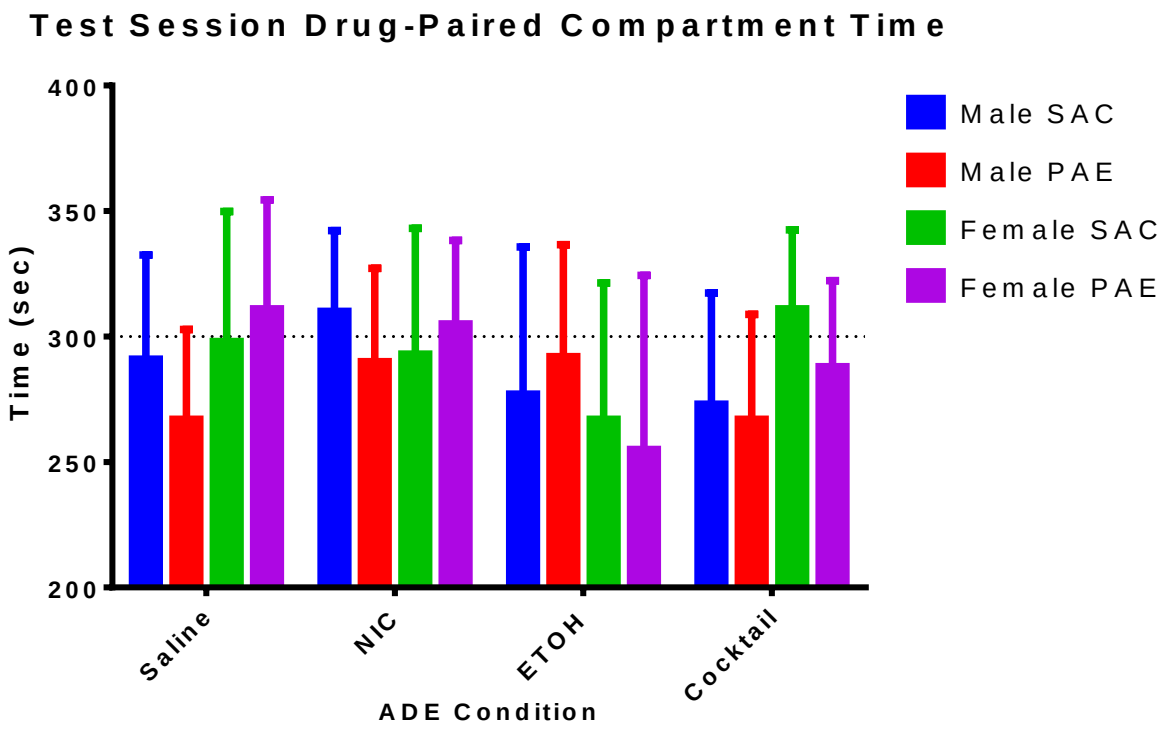
Error bars refer to 95% confidence intervals for the group mean. The dashed line is the grand mean of the distance travelled during saline conditioning sessions (2167 cm). SAC is the saccharine control condition during prenatal alcohol exposure. PAE is the prenatal alcohol exposure condition. ADE refers to adolescent drug exposure conditions.

Figure 2



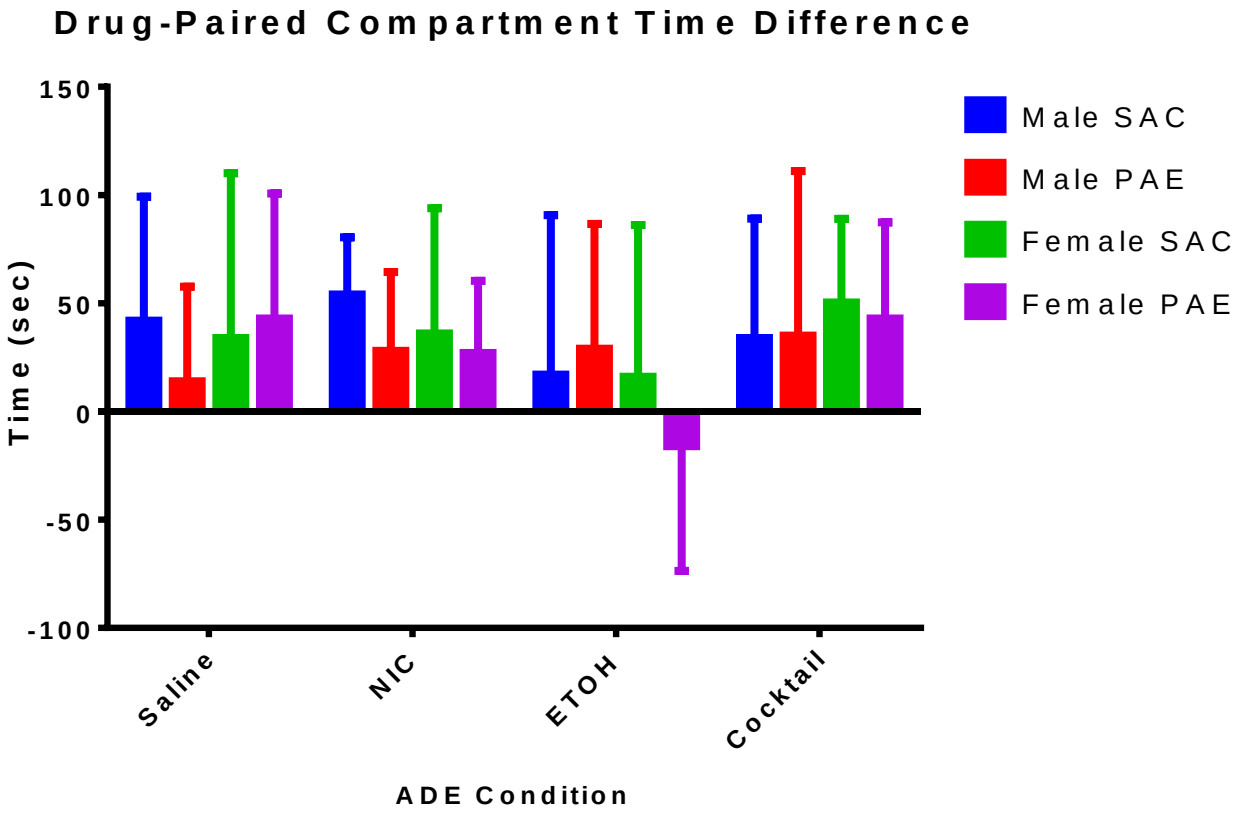
Error bars refer to 95% confidence intervals for the group mean. The dashed line is the grand mean of the distance travelled during saline conditioning sessions (2167 cm). SAC is the saccharine control condition during prenatal alcohol exposure. PAE is the prenatal alcohol exposure condition. ADE refers to adolescent drug exposure conditions.

Figure 3



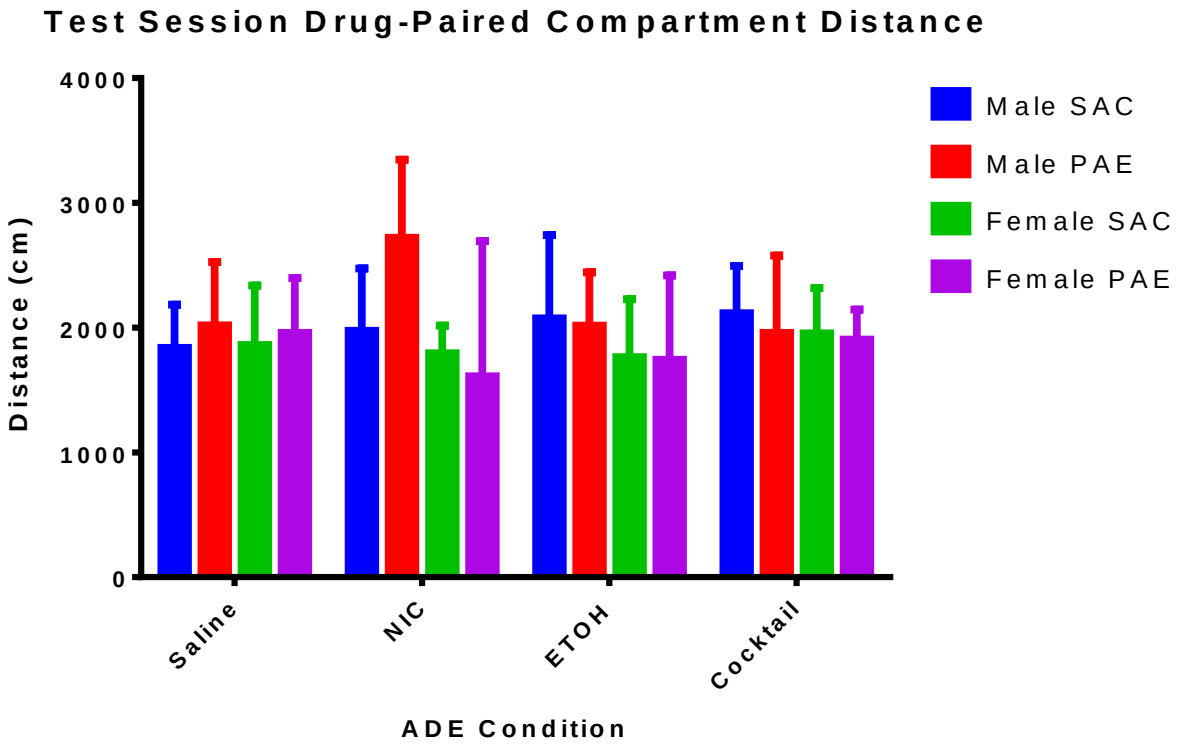
Error bars refer to 95% confidence intervals for the group mean. The dashed line refers to half of the time of the session (5 minutes). SAC is the saccharine control condition during prenatal alcohol exposure. PAE is the prenatal alcohol exposure condition. ADE refers to adolescent drug exposure conditions.

Figure 4



Error bars refer to 95% confidence intervals for the group mean. SAC is the saccharine control condition during prenatal alcohol exposure. PAE is the prenatal alcohol exposure condition. ADE refers to adolescent drug exposure conditions.

Figure 5



Error bars refer to 95% confidence intervals for the group mean. SAC is the saccharine control condition during prenatal alcohol exposure. PAE is the prenatal alcohol exposure condition. ADE refers to adolescent drug exposure conditions.